THE ACCELERATION OF THE RATE OF CELL DIVISION.[1]

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Cell division is a property of all animal and all plant cells upon which depends the ability of organisms to carry out progressive differentiation. While individual cells may grow and become specialized, a limit to the differentiation of the body cells is reached if division is lacking, a fact which finds illustration in the work on tissue cultures where the cells, though they may be maintained alive for protracted periods, do not greatly change in character, particularly if the mitotic ratio is low.

Differentiation in an organism consists of processes of several types. In an embryo the first differentiation processes must take place among the cells as units. Subsequently other processes begin within the cells themselves which result, for example, in the formation of contractile fibers in muscle cells, or in other well-known changes in cells of other kinds. These later processes do not usually begin, however, until the rate of division becomes differential. Conklin has defined differential division as follows: "Cell division is typical and non-differential when it occurs at regular intervals or at the same time in cells of the same generation (rhythmical) when successive divisions are at right angles (alternating), when daughter cells are of similar size (equal), and are composed of similar materials (homogeneous). Divisions are differential when they depart from these typical conditions in one or more respects, becoming non-rhythmical, non-alternating, unequal, or heterogeneous."

In an organism, then, the progressive differentiation of the cells must be thought of as cumulative, since in each cell generation the progress begins where it left off in the proceeding. The problem of the organization of the early embryo is largely one of the cumulative differentiation of the individual cells, of their increase

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in number, and in the complexity of the relationships which they assume to each other. Later the intracellular processes of specialization assume a larger importance. The rapidity of organization, therefore, is directly dependent upon the rate of division of the constituent cells of the embryo.

Differentiation, of course, is not synchronous with the division of any particular cells. In fact, growth, and the processes which accompany it, alternate in time with the successive mitoses. Laughlin obtained evidence of this fact in his investigations on the duration of the mitoses in the onion root tips. He pointed out that "in a growing tissue, so far as the individual cell is concerned, there is a definite alternation between permanent increase in bulk and mitosis. Indeed, if bulk increase is largely anabolic and cell division catabolic, as is most probably the case, then opposing activities can not synchronize in the same cell each as a dominant factor of activity. But synchronization of the same activities among many neighboring cells is a different matter. This exists and its degree determines the character of the pulsation observed in rate of growth in actively growing tissues." From the standpoint of a careful analysis, the processes which we usually speak of as growth are to be distinguished among themselves; mitosis, increase in bulk, and differentiation are all involved. Mitosis and increases in bulk can not usually take place in one cell at the same time. Their independence in this relation, however, does not mean that the three processes are not dependent on each other, for, of course, they must maintain their proper balance or the organism can not undergo further development.

That differential rates of division in the various organs and tissues of an embryo exist can easily be shown. A casual inspection of sections of any embryo will show that not all regions exhibit cells in division with equal frequency. The mitotic ratio (by which is meant the number of cells in some stage of mitosis that can be counted in one hundred cells of the type in question) varies within rather wide limits. From some investigations which have been going on in this laboratory it appears that the ratio for early chick embryos in their entirety varies from a fraction of 1 per cent. to about 5 per cent. In any embryo many division figures may be found in some sections, while in others almost none

occur. Stockard has made good use of the conception of differential rate of division in his recent paper which elaborates a hypothesis to account for the production of monsters upon this basis. Numerous investigators have reasoned that mitoses occur periodically, and that the duration of the periods may be influenced by various external factors. But these investigations leave unanswered many questions and raise many problems as to the rate of division that merit closer study. What is the normal rate of division for various tissues? What is the duration of each stage of mitosis? Do mitotic cycles go in waves? To what extent do all the cells of a given tissue divide simultaneously? If divisions are not synchronous, what changes in the mitotic ratio occur? What factors influence the rate of cell division? What are the limits of its modifiability?

One of the earliest attempts to study the rate of cell division resulted in the formation of the well-known "Balfour's law of cleavage." This law states in effect that the rate of cleavage is inversely proportional to the amount of deutoplasm that is contained within the dividing cell. Wilson's criticism of this law ("The Cell," p. 366) is all that is necessary to show that the formulation of it made no real addition to our knowledge of the subject. He said: "The entire inadequacy of this view has been demonstrated by a long series of precise studies on cell lineage, which show that while the large deutoplasm bearing cells often do divide more slowly than the smaller protoplasmic ones, the reverse is often the case, while remarkable differences in the rhythm of division are often observed in cells which do not appreciably differ in metaplasmic content. All the evidence indicates that rhythm of divisions is at bottom determined by factors of a very complex character which can not be disentangled from those which control growth in general. Lillie ('95, '99) points out the very interesting fact, determined through an analysis of the cell lineage of molluscs and annelids, that the rate of cleavage shows a direct relation to the period at which the products become functional. Thus in *Unio* the more rapid cleavage of a certain large cell ("D. 2"), formed at the fourth cleavage, is obviously correlated with the early formation of the shell gland to which it gives rise, while the relatively slow rate of division of the first ectomere quartet is

correlated with reduction of the pretrochal region. The prospective character shown here will be found to apply also to other characters of cleavage."

Throughout the literature of embryology one finds an occasional reference or allusion to the actual time that is consumed in the various early cleavages, but many of these are rather incidental and lack the character of systematic studies of the point. Of those in which information of real value is given, Conklin's notations on the rate of cleavage in *Cynthia* may serve as an example. The eggs of *Cynthia* were fertilized in a typical case at 5 o'clock P.M. and the development carefully followed through. The actual duration of the various stages was as follows:

Fertilization at 5 P.M. to first cleavage (1–2 cell)	40 min.
First to second cleavage (2–4 cell)	30 min.
Second to third cleavage (4–8 cell)	20 min.
Third to fourth cleavage (8–16 cell)	20 min.
Fourth to fifth cleavage (16–32 cell)	20 min.
Fifth to sixth cleavage (36–64 cell)	20 min.
Sixth to seventh cleavage (65–112 cell)	20 min.
Seventh to eighth cleavage (112–218 cell)	20 min.
Eighth to young tadpole.	2 hours.

Or, summarizing his observations, the period from fertilization to first cleavage is 40 minutes, from the first cleavage to the beginning of gastrulation 140 minutes, and again 140 minutes to the fully formed tadpole stage. This is rather rapid development, although in *Molgula,* another ascidian, the entire period is only two thirds as long.

The writer in 1914 recorded the average duration of the early cleavages of *Planorbis* eggs under average temperature conditions of the water of about 16–20° C. The durations of the periods required for the completions of the first, second, third, fourth, and fifth cleavages were, respectively, 1½ hours, 1¼ hours, 1¼ hours, 1¼ hours, and 1 hour.

For *Haminea virescens,* a typical case, recorded in connection with the experiments reported herein, gave the following figures for the durations of the first five successive cleavages: 85 minutes, 51 minutes, 59 minutes, 50 minutes, and 40 minutes.

In all these cases the actual duration of time differs, but the first cleavage is longer than those which follow it.

Somatic tissue cells have been followed through their divisions by a few investigators who have observed them either in growing mesenchyme of a tadpole tail (Clarke) or in tissue cultures (Lambert, Lambert and Hanes, Levi, and Lewis and Lewis). Clarke observed that the prophase and metaphase together lasted about 1 hour and 15 minutes, the anaphase 4 minutes, and the telophase 3 to 4 minutes (although complete separation of the cytoplasm followed much more slowly). Lambert and Hanes found in tissue culture that at 37° C. connective tissue cells of the cat divide in 15 to 30 minutes, while mitosis in similar cells of the rat last from 25 to 45 minutes. Levi studied tissue cultures from chick embryos and arrived at the following range of variation for the phases of mitosis therein: Prophase and metaphase together, 20 to 30 minutes, of which 8 to 13 minutes, according to further observations of his, were consumed by the metaphase; anaphases usually range from 3 to 7 minutes; telophases last from 1 to 10 minutes, although the great majority were from 3 to 6 minutes in duration. Lewis and Lewis made careful observations on the duration of various phases of mitosis in mesenchyme cells in tissue cultures and concluded that the time ranges for the various stages are as follows: prophase, 30 to 60 minutes; metaphase, 2 to 10 minutes; anaphase, 2 to 3 minutes; telophase, 3 to 12 minutes; and the reconstruction period, 30 to 180 minutes.

Laughlin has made the most extensive study of the duration of mitotic stages in dividing root tip cells of the onion. He employed

Stage Number.	Stage Description.	Average Duration, in Minutes.			
		At 10° C.		At 20° C.	At 30° C.
	Resting	194.92 min.		159.57	33.26 min.
1	Early prophase	52.2550	88.2693	59.2592	51.4147
2	Early prophase	22.1064		8.2376	1.6673
3	Mid prophase (spireme)	9.2036		3.1094	1.1776
4	Late prophase	4.7043		3.3943	1.2301
5	Metaphase	1.3859		.9849	.3212
6	Early anaphase	.6539	2.2109	.7651	.3264
7	Mid anaphase	.7222		.6267	.2273
8	Late anaphase	1.8348		1.1233	.4314
9	Early telophase (di-spireme)	2.0398	4.675	1.5303	.7054
10	Late telophase	2.6352		2.3443	.7579
	Totals	292.52		240.97	91.56

a detailed statistical treatment of a total of 55,000 cell counts, divided the mitotic process into ten stages whose limits were artificially defined, and determined the actual time of each stage at 10°, 20°, and 30° C. For the period of rapid elongation of the root tips the values for each stage are shown in the tabulated résumé.

Laughlin's observations upon the periodic character of mitosis and its rhythms have already been referred to.

The factors which govern cell division are not as yet clearly understood. In a matter of such vital character as mitosis and of such complexity it is not to be expected that clear conceptions will be easily forthcoming. One method by which an analysis may be attempted is that of modifying the process by various means. At the present time little in the way of reliable data is at hand to indicate to what extent or by what factors the rate of division may be modified.

There are many factors that will act to decrease the rate; in fact, any condition which is unfavorable to the growth and well-being of the organism, or which lowers its general metabolic activity, results in a decreased rate of division in tissues where multiplications are taking place. Some of these factors are definite and measurable in their results, as decreased temperature, while others are intangible and seem to affect the vitality of the entire organism rather than the mitotic mechanism. Because of this latter set of factors it is not profitable to attempt here a study of the causes of a lowered rate of multiplication.

On the other hand, the factors which can cause an increase in the rate of division are few, and must in the nature of the case work their effects directly upon the mechanism of mitosis. It is within the bounds of hope that their study will throw light upon this mechanism as well as upon the matter of differentiation and organization. Since little attention has been given to this matter by cytologists and experimental embryologists, it seems desirable to list the means by which increased division rates have been obtained and to point out at some length the nature of their effects.

The list of agencies by which acceleration of the rate of division may be accomplished is not a long one. One might infer that any

influence which will stimulate growth would also accelerate the division rate, but the evidence is not clear that such is always the case. For instance, Yung in 1881 exposed the eggs of *Limnæa stagnalis* to light of various wave lengths and found that the hatching periods varied from 17 days in the case of violet to 36 days in that of red; it would seem that the light must have accelerated the division rate, but since the data were not taken with this point in view, further evidence is necessary. The list of agencies in which the evidence is definite includes heat, x-rays, radium, thyroid secretion, supra-renal extract, alcohol, dibasic potassium phosphate, potassium sulphate, potassium bromide, oxygen, sodium hydroxide, and pilocarpine hydrochlorate.

That the rate of development of an organism depends upon temperature is a matter of common information. The development is retarded at low temperatures and increased as the temperature rises toward a maximum (above which, however, the rate almost instantly falls off until death results). The first careful inquiry into these matters was made by Oscar Hertwig ('96 and '98) upon the eggs of *Rana fusca*. Since Hertwig many investigators have studied the effects of temperature. The correlation between the increase in the rate of division and the rise in temperature has suggested that van't Hoff's law may apply to the mitotic processes. Mitosis, however, may not be looked upon as a simple reaction which would respond directly to temperature rises, so the results, as might be expected, have not been entirely uniform upon this point. Laughlin investigated the division rates at 10°, 20°, and 30° in his studies on the onion root tip and determined the values of Q_{10} (the velocity increase at a given temperature compared with the velocity of the same stage at 10° C. lower). He says: "From the Q_{10} values derived from these comparisons it is found that each mitotic stage presents characteristic velocity reactions to temperature increments. These reaction values approximate van't Hoff's expectations, thus indicating that most probably the repertoire of activities constituting each stage is composed of the actions and interactions of those much more elementary physical and chemical forces which measured in more isolated relations have been shown to react in this same velocity fashion."

The acceleration of the cleavage rates of eggs, due to their

exposure under certain conditions to x-rays and radium rays, are perhaps the most marked which any of the stimulating agents are able to bring about. The effect of the radium rays upon division rates have been studied by Hertwig, Packard, and others. Packard exposed *Arbacia* eggs to radium rays and noted that "A short radiation brought about a stimulation; while a longer one produced a retard. Between these two limits there was a strength of radiation which produced no noticeable effect—*i.e.*, the initial acceleration was overcome by a subsequent retard." He argues that these results were brought about because the radiation thus effects the enzymes of the cells. These conclusions are confirmatory to a preliminary experiment of Lazarus-Barlow and Bonney and to subsequent extensive research by Lazarus-Barlow and Beckton. Since these two latter papers appeared in a publication not generally accessible to zoölogists in this country, it seems worth while to quote the conclusions in full:

"If radium act on ova of *Ascaris megalocephala* in the resting stage in quantities of the order 5×10^{-7} mgr. and for a continuous period of about 30 hours at 0° C., cellular division subsequently proceeds at an accelerated rate.

"Greater quantities than the above or more prolonged exposures progressively retard the rate of division.

"These effects are brought about by the action of alpha, beta, and gamma rays acting together.

"Beta and gamma rays alone (alpha rays being excluded) act similarly.

"The action of the alpha rays appears to be about one hundred times as great as the action of the beta rays."

Packard, Lazarus-Barlow and Beckton, and Mottram all agree that the acceleration is greater if the exposures are made in the dividing stages of nuclei rather than during resting periods. Mottram concludes "that the animal cell, as exemplified by the ova of *Ascaris*, is at least eight times as vulnerable to the beta plus gamma rays of radium in the dividing as in the resting stage of its nucleus; and, further, that this increased vulnerability during division concerns the metaphase." This general conclusion that eggs are more susceptible during the height of the division stages

is one that is familiar to experimental embryologists, having been demonstrated with many eggs and many agencies.

Bohn found that an exposure to radium of forty minutes accelerated segmentation in eggs of the sea urchin, although a longer exposure retarded it.

The use of x-rays agrees in most respects with that of radium. Gilman and Baetjer exposed hen's eggs for ten minutes daily to x-rays. During the first thirty-six hours the development was accelerated. Then there followed a retardation during which the development was greatly altered as well as checked. Comparable results were obtained by these same investigators working on the eggs of *Amblystoma*. Exposures of fifteen minutes daily first produced a period of acceleration which lasted up to ten days in some embryos, but at the end of the fourth day abnormalities began to manifest themselves.

While investigating the effect of x-radiation on *Planorbis* eggs the writer found convincing evidence of their ability to accelerate cleavage. The eggs normally require from fifty-five minutes to two hours to complete a division (up to the 24-cell stage). The first effect of exposure of not to exceed ten minutes, if made during the formation of the mitotic spindle, is to accelerate the division of the egg. Even a very short stimulation will produce this phase of acceleration, which is then followed by a phase of depression; the end result is to retard greatly the development of the egg. The acceleration of the rate of cleavage in *Planorbis* as a result of exposure to x-radiation is the most extreme response of this character with which the writer is acquainted. "This effect was first obtained after eggs had been exposed ten minutes, when it was noticed that divisions had actually been completed in cells where only a spindle was to be seen at the time the exposure began—that is, during an exposure of ten minutes there had been accomplished a complete process which never under normal conditions had been observed in this form to occur in much less than an hour. I have repeated this observation from January to June on many experiments and have obtained the result without variation. Whenever an egg of *Planorbis* in any cleavage up to the sixth, farther than which it is not practical to carry on observations on the living egg, is exposed to x-rays any mitosis which may have

been started is hastened to completion, and in almost every case that state has been reached by the time the egg can be taken from under the tube and examined under the microscope." During the depression phase following exposure of this egg a second stimulation may be brought about by reëxposure, but the extent of the acceleration is less and the second depression follows more rapidly.

A later experiment of slightly different character, but bearing, I think, upon this general problem, may be noted here. The writer exposed pepsin and diastase to x-rays and found that a short radiation accelerated the activity of both these preparations of enzymes, that a longer exposure inhibited the activity, and that between these two strengths there lies a point at which radiation is non-effective. Exactly similar results were obtained by the writer and Miss Woodward upon exposing the cell extractive of Echinoderm eggs, "fertilizin," to x-radiation. The parallel between the behavior of these enzymes and extractives and that of mitotic processes under the influence of radiation is probably not without its significance.

Certain substances derived from internal glands are thought to favor growth, chief among them being thyroid constituents. It might be presumed from this effect that these substances would also stimulate cell division. Nowikoff, Shumway, and also Buddington and Harvey have investigated the effect of thyroid upon ciliates and have arrived at essentially the same conclusion. Shumway added a small amount of thyroid emulsion to the hay infusion in which *Paramœcium aurelia* were growing and a sharply marked increase in the division rate resulted. He reports as follows: "Thyroid substance fed to *Paramœcium aurelia* or *caudatum*, either as an emulsion of raw thyroids or as a suspension of the commercial powder, produces a constant and significant increase of 65 per cent. in the rate of division over that observed in the common laboratory hay-medium infusion. The thyroid is the only one of the internally secreting glands that produces this effect. Boiling the thyroid produced no change in the reaction. Iodothyrin and iodine fail to produce the thyroid effect. *Paramœcia* after prolonged thyroid treatment revert to the normal division rate when returned to the control medium. The life-history curves of the thyroid-treated lines show the same depression

periods at the same time intervals as the control lines, and thyroid produces the greatest acceleration of the division rate when the control line is dividing most rapidly." In one experiment, however, the use of thymus, adrenal, and pituitary substances also gave an acceleration of the rate of division, but other experiments did not confirm these data.

In experiments which are somewhat similar to those of Shumway, Miss Chambers obtained results which indicate that the division rate of *Paramecia* was increased by the feeding of ground yeast, of pituitary preparation, and especially of suprarenal solution. In the latter case "these results are more constant than those obtained by feeding pituitary solution. There is a slight but decided increase over the control lines."

Paramœcium has been the subject of a number of experiments of this sort, for it has responded by alterations of its division rate to several kinds of stimuli. Calkins and Lieb found that one part of alcohol in about 2,500 parts of culture medium acted continuously to accelerate the division rate. Woodruff has shown that the effect of small amounts of alcohol on *Paramœcium* and *Stylonychia* as well is to produce a much more rapid rate of division in the experiment than in the control during the first month of the work, but after that the rate decreased as compared with the control, and this is followed by fluctuations both above and below the control. Woodruff had earlier shown that dibasic potassium phosphate (K_2HPO_4) caused an acceleration of the rate of division during the early part of the cycle of an *Oxytricha fallax* culture, and a retardation during the latter part. Potassium sulphate (K_2SO_4) and potassium bromide (KBr) in $n/100$ solutions likewise caused slight acceleration. The stimulating effect of the treatment gradually wears off and the agents become depressants, but an increased dose will again cause a stimulation. The temporary effect of alcohol upon the rate of division of *Paramœcium* reminds the writer of his own observations on the effect of x-rays upon the rate of cleavage of *Planorbis* eggs.

The effect of oxygen upon developing eggs has been studied by a number of investigators with various problems in mind. In one case results were obtained which allow conclusions to be drawn as to the effect upon the rate of division. Godlewski found that

an atmosphere of pure oxygen accelerates the development of the eggs of *Rana temporaria*. He subjected eggs to oxygen, hydrogen, and to oxygen and carbon dioxide. The first one gave marked evidence of acceleration, as follows (table from Jenkinson):

Hours.	Oxygen.	Controls.
3	First furrow in some.	No furrow.
3½	All but one with first furrow.	Most with first furrow.
4	All but one four cells.	All with two cells.
5	All with four cells.	Most with two cells; a few with four.
47	Blastopore closed	White hemisphere visible.
73	Medullary fold.	Blastopore closed.

The experiments were carefully done so that matters of pressure and other disturbing factors were controlled, and the results may be attributed to the factor under consideration. The data from the experiments with hydrogen seem less conclusive as to the effects upon rate of cleavage, while no segmentations were obtained in the carbon dioxide experiments.

The effect of sodium hydrate upon *Arbacia* eggs was studied by Loeb. He found that the development and growth can be accelerated if the solution be made weakly alkaline, the concentration of sodium hydrate used being very small, perhaps .006 per cent. to .008 per cent. Acids have only an inhibiting effect. The chief cause for these effects must be that the oxidative processes in living substance are favored by the weak alkali, while acids decrease the oxidative processes and thereby inhibit syntheses. Loeb found that more than .2 cubic centimeter of 1/10 normal NaOH would not go into solution in 100 c.c. of sea water, for the amount of precipitate formed was only increased. Cleavage in the alkaline solution was slightly accelerated, but the amount of the increase was difficult to detect for any particular stage; as development progressed the effects became more clearly recognizable, and by the time the swimming blastula stage is reached the difference had become so pronounced that movement clearly began earlier in the eggs in the alkaline solution. In one case fertilization took place at 9:30 and at 3:15 the embryos in the alkaline sea water were swimming in lively manner, while the embryos in normal sea water remained motionless. In another

case fertilization was at 11:00, 50 per cent. of the embryos in the alkaline sea water were swimming at 4:20, although all were motionless in the normal, and the first signs of movement in the latter were seen at 4:40. Loeb showed that the effects of the alkali were not simply upon ciliary movement, but also upon the development of the eggs themselves, for the next morning after such an experiment as the one just described the embryos in the alkaline solution would be in the *pluteus,* for example, and the normals in the round gastrula stage. And the alkali produced a difference not only in acceleration of the rate, but also an increase in the size of the plutei.

A similar action to that of the hydroxyl-ions was obtained by Mathews by the employment of pilocarpine hydrochlorate on *Asterias* larvæ. He found that the pilocarpine hastens the development and gives rise to abnormally large embryos, while atropine hindered the development and gave rise to dwarf embryos. Both of these drugs act directly upon the animal cells, and the nature of their action suggests that the atropine inhibits the oxidations taking place in those cells, while the pilocarpine increases those oxidations.

All of these agencies have the effect of accelerating the rate of cell division. In most cases the effect is only a slight one, but some of the stimulants are marked in their efficiency. However, even slight effects are significant if they are constant, and a careful analysis of the means by which acceleration even of slight amount may be produced will doubtless give additional light on the mechanism by which cell division is brought about.

Experiments with *Haminea* Eggs.

During the summer of 1921 the writer studied the effect of a number of accelerants upon the cleavage of the eggs of the opisthobranch *Haminea virescens* (Sowerby) at the laboratory of the Scripps Institution for Biological Research at La Jolla, California. The eggs of this animal are particularly favorable for experiments in which it is desired to test the effect of some special factor while leaving the egg in an environment that is normal in all respects except that investigated. The animals were brought into the laboratory and kept in a dish of running sea water supplied with a

quantity of stones and sand from the tidal flat where they were first secured. Although they would at length become exhausted, no difficulty was experienced for some days in getting them to produce eggs.

The eggs are laid in a jelly mass which has the appearance of a short piece of narrow but very thick ribbon. It is of rather complicated structure. The eggs appear to be extruded in a string of tough gelatinous material which becomes surrounded by the matrix jelly forming the body of the ribbon. The string itself is laid in a zigzag fashion, so that the appearance is that of a double row of eggs. It is, however, accurately placed in the form of a flattened spiral so that the loops are not formed by simple back and forth folds as they at first appear, but are so arranged that the loops are compressed against each other. This produced the effect of a thick cross-striated ribbon. In one typical ribbon 242 loops were counted, in each of which the eggs averaged 90; this gave a total of 21,780 eggs for this ribbon. Probably 20,000 is an average number for a ribbon produced under typical conditions.

In each ribbon the eggs are uniformly all in the same stage of development, indeed in the same stage of mitotic division. It is a remarkable fact that 20,000 eggs should be deposited in as complicated a manner as these and all be in the same stage of division. But it is this fact in connection with the ribbon-like egg case that renders them desirable for experimental purposes. In conducting the experiments a ribbon would be cut into segments, one or more of which would form a control, while the others would be placed in the various solutions as desired and the results noted in comparison to the control.

The purpose of the first experiments upon these eggs was to verify and to extend Loeb's observations on *Arbacia* eggs that a small amount (.006 per cent.) of sodium hydrate would accelerate development. A considerable number of experiments planned to determine the effect of various concentrations of NaOH on the eggs were carried on. The general results of these were two. Some acceleration of cleavage resulted if the eggs were allowed to develop in sea water containing from .004 per cent. to .009 per cent. NaOH. Acceleration of cleavage does not always result in an earlier hatching of the larvæ, for it would seem that the advan-

tage gained in the early stages sometimes takes expression in more vigorous larvæ rather than in more rapidly developing larvæ. In some experiments, indeed, hatching seems actually to be delayed by the treatment. A summary of the experiment numbered 156 is here given as an example of the sodium hydrate effect. Many other experiments were performed to verify these points, but they need not be given here.

Experiment No. 156. July 26, 1921. Began at 6:50 A.M. Eggs in 4-cell Stage.

6:50 A.M.	7:25 A.M.	8:45 A.M.	9:20 A.M.	12:00 A.M.	2:30 A.M.
156.1 Control (10 c.c. sea water)	4 cell	12	12–16	20	24–28 cell
156.2 0.2 c.c. 1/100 per cent. NaOH (in 10 c.c. sea water)	4–8	12	12–16	20	24–28 cell
156.3 0.4 c.c. 1/100 per cent. NaOH (in 10 c.c. sea water)		12	16	20–24	32–36
156.4 0.8 c.c. 1/100 per cent. NaOH (in 10 c.c. sea water)	4–8	12–16	16–20	20–24	32–36
156.5 1 c.c. 1/100 per cent. NaOH (in 10 c.c. sea water)	4–8	12–16	16	20	28

7/29, 10:00 A.M. 156.1 Larva large, moderately active.
156.2 Larva active, rotating.
156.3 Larva active, healthy.
156.4 Larva active, rotating.
156.5 Dead.

The effect of ammonium hydroxide is seen in experiment 159, for example. Here, too, the acceleration is evident. It is a little more difficult to obtain accurate results with ammonia since the concentration of the solution changes. I think, however, that the effects are not different in character from those of the sodium hydrate, although they are perhaps less evident in this case.

Experiment No. 159. July 31, 1921. Began at 7:10 Eggs Just Completed 3rd. Division.

7:10 A.M. Early 8 cell.	7:50 A.M.	9:20 A.M.	10:25 A.M.	11:50 A.M.
159.1 Control (10 c.c. sea water)	8 cell	16	20	28–32
159.3 0.6 c.c. 1/100 per cent. NH_4OH (in 10 c.c. sea water)	8	16	20–25	32
159.4 0.9 c.c. 1/100 per cent. NH_4OH (in 10 c.c. sea water)	8	12–16	20–24	32–36
159.5 1.2 c.c. 1/100 per cent. NH_4OH (in 10 c.c. sea water)	8	12–16	20	32

Potassium hydroxide is also similar in its effect, but the eggs respond with the more rapid division rate only to a slightly stronger concentration than in the case of NaOH. Experiment 161 furnishes an example of the KOH effects.

EXPERIMENT NO. 161, AUGUST 2, 1921. BEGAN AT 7:00 A.M. EGGS IN 4-CELL STAGE.

7:00 A.M. 4 Cell.	7:45 A.M.	8:45 A.M.	9:40 A.M.	10:30 A.M.	12:15 A.M.
161.1 Control (9 c.c. sea water)	8	12	16	20	24–28
161.2 0.3 c.c. 1/100 per cent. KOH (in 10 c.c. sea water)..	8	12	16	20	24–28
161.3 0.6 c.c. 1/100 per cent. KOH (in 10 c.c. sea water)..	8	12	16–20	24	28–32
161.4 0.9 c.c. 1/100 per cent. KOH (in 10 c.c. sea water)..	8	12	20	24	32
161.5 1.7 c.c. 1/100 per cent. KOH (in 10 c.c. sea water)..	8	12	20	24	24–28

8/8 7:00 A. M.	8/9 6:45 A. M.
161.1 ¾ hatched.	all hatched.
161.2 2/3 hatched.	8/10 hatched.
161.3 9/10 hatched.	all hatched.
161.4 A few hatched.	never hatched.
161.5 All hatched.	

It was desired by the writer to try the effect of various other hydroxides, especially those belonging to the first periodic group; but in the time at his disposal for the experiments he was able to obtain only two others, namely, barium hydroxide, $Ba(OH)_2$, and chromium hydroxide, $Cr_2(OH)_3$. Eggs were placed in solutions containing, respectively, .005 per cent., .006 per cent., .009 per cent., and .01 per cent. of barium hydroxide. In one experiment it seemed that some slight acceleration of the cleavage rate was probably affected by the reagent, but it was not possible afterwards to repeat that result and the writer is therefore disposed to believe that some other factor must have been responsible for the response observed. With chromium hydroxide also no acceleration could be seen in the cleavage rate.

Several experiments upon the effect of thyroid extract upon the cleavage rate were tried. A preparation of Parke Davis's desiccated thyroid was tried, with somewhat varying results. The experiments justify the statement that thyroid contains some agent which is able to increase at least slightly the rate of cleavage in

these eggs, but the nature of the desiccated gland and the conditions of the experiments do not lend themselves to an exact statement of the effect itself or of the strength of material necessary to produce it. It is to be regretted that there was no further opportunity to study this matter in detail upon *Haminea* eggs.

Finally, influenced by Mathews's finding that the development of *Asterias* larvæ is hastened by pilocarpine hydrochlorate, the writer investigated the effect of this reagent upon *Haminea*. Mathews did not record any direct observations on the rate of cleavage, although an acceleration is to be inferred from his general results. The effect of pilocarpine on *Haminea* eggs was tried on concentrations of .1 c.c., .2 c.c., .3 c.c., .4 c.c., and .5 c.c. of ½ per cent. solution in 10 c.c. sea water. It was found that for these concentrations the effect, although slight, is an acceleration in any one cleavage in proportion to the concentration. The effect is less marked than in the case of the hydroxides of the first periodic group. Experiments 172.1 and 172.4 will serve as examples of the effects of pilocarpine hydrochlorate treatment. Pilocarpine nitrate was also tried, but the acceleration was not obtained by its use. It is probable that it is only the hydrochlorate which possesses the property of accelerating division rate.

Experiment No. 172. August 16, 1921. Began at 6:35 A.M. Eggs Just Completed 2nd Division.

6:35 8 Cell.	7:05 A.M.	7:55 A.M.	9:50 A.M.	8/18.
172.1 Control (10 c.c. sea water).	8	12–16	24	Rotating slowly.
172.4 0.3 c.c. ½ per cent. pilocarpine in (10 c.c. sea water)...	8–12	16	24–28	Rotating, some rapidly.

Discussion.

These experiments have raised a number of questions that the writer was unable to investigate because of the shortness of time at his disposal. In continuing the work it is necessary to use other material than *Haminea* eggs, a condition which is to be regretted, since these eggs are so favorable for this study. Work is nevertheless going on in this laboratory at the present time in an attempt to extend and develop certain of these conclusions and to clear up some of the questions.

If the list of hydroxides which cause acceleration be inspected, it is noted that they are the hydroxides of elements which belong to the first group of the periodic series (with the exception of ammonium, which, however, behaves chemically as do the members of that group). It is to be noted also that the hydroxides of barium of the second group and of chromium of the sixth group fail to induce the acceleration. One may suppose that it is only hydroxides of the first group that are effective in causing acceleration. This tentative conclusion is the object of certain experiments now being carried on, which at the date of the writing seem to bear it out. It is true that only hydroxides of the first group have any high degree of solubility, but this is discounted by the fact that the amount of any hydroxide which will go into solution in the already slightly alkaline sea water without causing precipitation is very small.

These results, I think, throw light upon the nature of the mitotic process and upon the mechanism by which it is accomplished. Practically all of the means now at our disposal for accelerating the rate of mitotic divisions are in line with increased oxidations and increased metabolism of the cells. Furthermore, a number of these accelerating agents are already known to affect favorably enzymatic activity. For example, the radium and x-ray studies previously referred to indicate that the accelerating and retarding effects of radiation are produced upon the enzymes of the cells. In this connection it is to be recalled that Mathews and others have suggested that the mitotic processes are correlated with the setting free and the activation of intracellular enzymes. The changes in the rate of enzyme action are able to bring about changes in the rate of division; and since the radiations affect the enzymatic activity, it is to be supposed that the changes which they induce in the rate of division are to be accounted for on the basis of this chemical mechanism. These considerations lead the writer to suggest that the agents which have been shown (either in this paper or in the list on a preceding page) to be capable of accelerating division do so because they possess the property of activating, either directly or indirectly, the inactive enzymes of the cells, or by increasing the activity of those which have already been activated. This line of approach offers a most profitable opportunity,

it would seem, for the analysis of the forces and mechanism of mitosis.

Summary.

The list of agencies which have been previously found to accelerate the rate of cell division includes heat, x-rays, radium, thyroid secretion, suprarenal extract, alcohol, dibasic potassium phosphate, potassium sulphate, potassium bromide, oxygen, sodium hydroxide, and pilocarpine hydrochlorate.

The results of the experiments on the eggs of *Haminea virescens* are in harmony with those of previous investigators. These experiments show that the eggs may be induced to undergo cleavage at an accelerated rate (although the amount of the acceleration need not be great) by the following reagents: .004 per cent. to .009 per cent. NaOH, .006 per cent. to .009 per cent. NH_4OH, .006 per cent. to .017 per cent. KOH, thyroid extract and pilocarpine hydrochlorate in weak concentrations. The accelerating effect of the pilocarpine is less than that of the hydroxides mentioned.

Barium hydroxide, chromium hydroxide, and pilocarpine nitrate cause no acceleration.

The experiments suggest that the hydroxides which are effective in causing acceleration of cleavage are those of elements which belong to the first group of the periodic series, and that those of other groups are ineffective in these respects.

Acceleration of cleavage is not always followed by continuously quickened development, for in some cases the experimental eggs do not hatch ahead of the control. The advantage gained in the segmentation stages may later manifest itself in more vigorous larvæ rather than in more rapidly developing ones.

The conclusion is also suggested that the agencies which are capable of accelerating division bring about this result through their property of influencing the enzymes of the cells, the setting free and the activation of which are correlated with the mitotic processes.

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